Science Together

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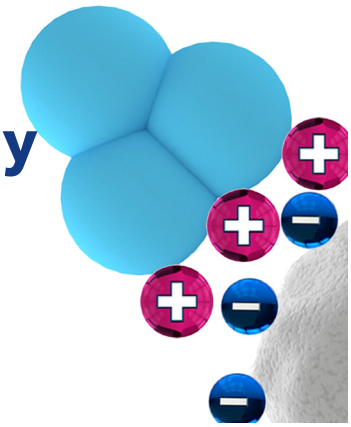
Separation of proteins with cation exchange chromatography on Sepapure SP and CM



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SUMMARY

Ion exchange chromatography is one of the most widely used FPLC techniques for protein separation and purification. Depending on the charge of the sample and the resin cation or anion exchange chromatography is used. This application describes an easy separation of model proteins and explains how cation exchange chromatography works.

INTRODUCTION

Ion exchange chromatography separates molecules according to type and strength of their charge. The isoelectric point (pI) is the pH where a protein or molecule has no net electrical charge. Depending on the pH of the buffer a protein has different surface charges in solution. At a pH below their pI proteins have positive charge and bind to negatively charged cation exchangers **Fig 1)**. This interaction is used for the separation and purification of various proteins. By using a suitable pH and low salt conditions proteins bind to the resin in the initial step. Proteins are mostly separated with a linear salt gradient whereby the salt ions compete with the proteins for bindings sites. Proteins with weak ionic interactions are the first to elute from the column. In the case of cation exchange chromatography, proteins that are less positivly charged start to elute first. With an increase of the salt concentration proteins with stronger ionic interaction elute later from the column. Ion exchange resins are categorized as strong or weak exchangers. Strong ion exchange resins are fully charged over a wide range of pH levels, while weak ion exchangers have depending on the pH varying ion exchange capacity. Weak ion exchangers have different selectivity's compared to strong ion exchangers. This application describes the separation of Cytochrome C, Lysozyme, and Ribonuclease A on a weak and a strong cation exchanger and explains the principle of cation exchange chromatography.

Additional Information

Separation of proteins with cation exchange chromatography on Sepapure SP and CM

RESULTS

Cytochrome C (pI 10.3), Lysozyme (pI 11.35), and Ribonuclease A (pI 9.6) are proteins with relatively high pI values, which make them ideal candidates for cation exchange chromatography **(Fig 2)**. All three proteins bind under low salt conditions to the resin. Ribonuclease A eluted first from the column due to its lower pI of 9.6 **(Fig 2**, peak 1**)**. With an increasing gradient and therefore increasing salt concentration Cytochrome C eluted as second peak while Lysozyme eluted as third peak. The identical protein mix was run on a weak (light blue signal Sepapure CM) and strong (dark blue signal Sepapure SP) cation exchangers showing the different selectivity of these two resins.

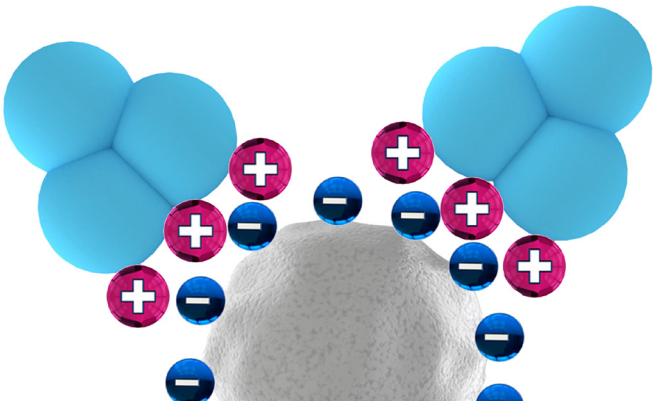


Fig. 1 Principle of cation exchange chromatography

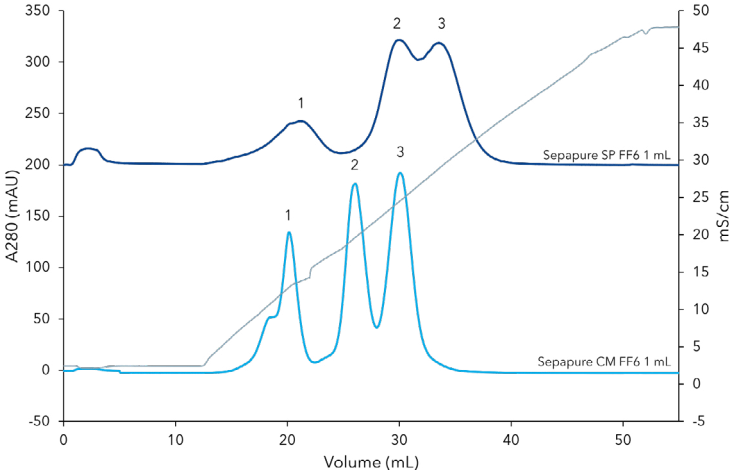


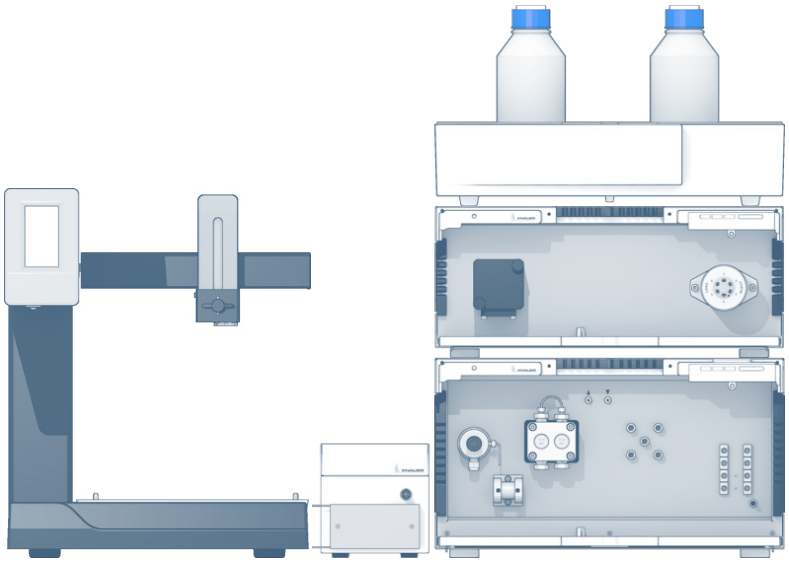
Fig. 2 Chromatograms of the separation of Ribonuclease A (1), Cytochrome C (2), and Lysozyme (3) with weak (light blue line) and strong (dark blue line) cation exchange chromatography columns, grey line: conductivity signal

MATERIALS AND METHODS

In this application, an AZURA® Bio purification system consisting of AZURA P 6.1L LPG metal-free pump with 10 mL pump head; AZURA ASM 2.1L assistant module with an injection valve and a single wavelength UV detector UVD 2.1S; AZURA CM 2.1S conductivity monitor and Foxy R1 fraction collector was used. Cytochrome C (0.4 mg/mL), Lysozyme (0.4 mg/mL) and Ribonuclease A (1 mg/mL) were diluted and mixed in buffer A (20 mM Sodium phosphate buffer pH 6.8) to the final concentration. Prior to the run the cation exchange columns (Sepapure SP FF6 1 mL and Sepapure CM FF6 1 mL) were equilibrated with buffer A. 2 ml of the sample was injected with a flowrate of 1 ml/min. The column was washed with 10 column volume (CV) of buffer A to remove all unbound protein. The proteins were eluted with a linear gradient over 40 CV up to 50 % buffer B (20 mM Sodium phosphate buffer pH 6.8, 1 M NaCl). The proteins were detected at 280 nm and conductivity signal was recorded to monitor the salt gradient.

CONCLUSION

Three model proteins with different surface charges eluted under increasing salt concentrations from the cation exchange columns illustrating the principle of cation exchange chromatography. The application demonstrates the different selectivity of Sepapure CM, a weak, and Sepapure SP, a strong, cation exchange column.



ADDITIONAL MATERIALS AND METHODS

Tab. A1 Method parameters

Eluent A	20 mM Sodium phosphate buffer pH 6.8		
Eluent B	20 mM Sodium phosphate buffer pH 6.8 + 1 M NaCl		
Gradient	Volume [mL]	% A	% B
	10 step	100	0
	40 gradient	50	50
	5 step	50	50
	10 step	100	0
Flow rate	1 mL/min (2 mL/min from 50 mL)	System pressure	>3 bar
Run temperature	RT	Run time	57.5 min
Injection volume	2 mL	Injection mode	Automatic injection valve
Detection UV	280 nm	Data rate	2 Hz

Tab. A2 System configuration

Instrument	Description	Article No.
Pump	AZURA P6.1L, LPG 10 mL PEEK PK	APH69EB
Assistant	AZURA ASM 2.1L Right: UVD 2.1S Middle: - Left: V2.1S 6 Port/6 Position PEEK 1/16"	AYCALXEC
Flow cell	3 mm semiprep, 2 µL, biocompatibel	A4045
Conductivity monitor	AZURA CM 2.1S	ADG30
Flow cell	Preparative up to 100 mL/min	A4157
Column	Sepapure SP FF6 1ml Sepapure CM FF6 1ml	010X15RSPZ 010X15QSPZ
Fraction collector	Foxy R1	A2650
Software	Purity Chrom	A59100

RELATED KNAUER APPLICATIONS

VBS0070 – Ion Exchange Chromatography with AZURA® Bio purification system

VBS0071 – Comparison of two column sets for antibody purification in an automated two step purification process

VBS0073 – Separation of proteins with anion exchange chromatography on Sepapure Q and DEAE

VBS0074 – Comparison of ion exchange columns